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Biochemical Changes in Humans upon Exposure to Sulfuric Acid Aerosol and Exercise

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16. ABSTRACT

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Biochemical Changes in Humans upon Exposure to Sulfuric Acid Aerosol and Exercise

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ABSTRACT. This study evaluated the effect of a single exposure to sulfuric acid aerosol on six human biochemical blood parameters. A total of 18 human subjects were exposed to ambient air for 4 hr on the first day of exposure and to 100 $\mu g/m^3$ (0.033 μM) sulfuric acid aerosol (0.5 μm mean mass diameter) for 4 hr on the second day. A total of 17 human subjects were exposed for 4 hr to ambient air on both exposure days. On each exposure day, at 30 min and 90 min after exposure was initiated, all subjects engaged in a 15-min exercise period (walking 4 mph on a treadmill inclined at 10°). Six biochemical blood parameters were measured before and after exposure: glutathione, lysozyme, glutathione reductase, serum glutamic oxaloacetic acid transaminase, serum vitamin E, and 2, 3-diphosphoglyceric acid. The results indicate no significant effect of one 4-hr exposure of humans to 100 $\mu g/m^3$ sulfuric acid aerosol. One significant post-exposure effect did occur—an increase in glutathione reductase—for both the control and acid-exposed group. It is concluded that at the levels studied, sulfuric acid aerosol is neutralized in the upper airways; consequently, no cellular damage is measurable.

STUDIES HAVE SHOWN that sulfuric acid (H_2SO_4) and particulate sulfates are formed by the oxidation of a portion of the sulfur dioxide (SO_2) emitted into the atmosphere.[1] Experimental toxicology studies have suggested that these oxidation products have a greater irritant potency than SO_2 gas per se.[2] Human H_2SO_4 exposure studies have concentrated mainly on the retention of inhaled acid mist as a function of particle size, and the concentration and magnitude of the response as measured by pulmonary function effects.[2-6] Few studies have evaluated the effects of H_2SO_4 mist on biochemical blood parameters. Alarie et al.[7] exposed cynomolgus monkeys and guinea pigs to H_2SO_4 mist for 78 wk and 52 wk, respectively. No deleterious effects due to H_2SO_4 mist on the measured biochemical blood parameters could be detected. Since H_2SO_4 is a part of our modern environment, it is necessary to establish if any responses are observed in humans under controlled exposure conditions. This study was designed to further define the possible irritant effects of H_2SO_4 mist on human health. Six biochemical blood parameters were evaluated in humans exposed to H_2SO_4 mist.

METHODS AND MATERIALS

Subjects. A total of 35 healthy (as determined by a physical examination and completion of the Duke University Computer History form and the Minnesota Multiphasic Personality Inventory), nonsmoking, Caucasian, male, university students were used for this study. All males were characterized by an average height of 179.8 cm [standard deviation (*SD*) = 7.2 cm; range = 160.0-195.0 cm], an average weight of 72.6 kg (*SD* = 8.6 kg; range

= 56.6-95.5 kg), and an average age of 28.0 yr (*SD* = 3.5 yr; range = 21.7-34.4 yr). A total of 17 subjects were exposed to air only [Group 1 (controls)], and a total of 18 subjects were exposed to H_2SO_4 aerosol [Group 2 (experimental)]. All participants were informed of any risks involved in the study, and each signed an informed consent statement with the understanding that withdrawal at any time during the study would be allowed.

Procedure. Subjects who participated in the study were tested in groups of three for 2 days. On each day blood was drawn immediately preceeding and following a 4-hr exposure to aerosols. During the exposure a pulmonary function battery including function residual capacity, airway resistance measured plettysmographically, forced inspiratory volume, forced vital capacity, vital capacity, and maximum voluntary ventillation, was administered at 0, 2, and 4 hr. This data, which showed no significant effects of the exposure, will be reported in a separate manuscript. Minute ventilation was recorded during the first 15 min ventilation was recorded. At 30 min and 90 min after exposure was initiated the subjects engaged in a 15-min exercise period that consisted of walking 4 mph on a treadmill inclined at 10°.

Experimental design. All subjects were exposed to ambient air on the first day of exposure and 18 of the subjects were exposed to 100 $\mu g/m^3$ (0.033 μM) H_2SO_4 aerosol [0.5 μm mean mass diameter (MMD)] on day 2. The remaining 17 subjects received ambient air on the second exposure day. Data were collected in a counterbalanced fashion. During week one, a group of three H_2SO_4-exposed subjects was tested on Monday and Tuesday, and a group of three air-exposed subjects was tested on Wednesday and Thursday. The following week the order was reversed. Exposures began at approximately 8:30 A.M. each morning. This alternation scheme was continued throughout the experiment, which helped to control any "day-of-week" effects and "time-of-year" effects.

The chamber atmosphere was maintained at a temperature of 22°C, a relative humidity of 40%, and an air flow of 227 m^3/min.

A total of six serum and red blood cell (RBC) biochemical measures were chosen as dependent variables: (1) serum glutathione (GSH), (2) lysozyme, (3), 2,3-diphosphoglycerate (2,3-DPG), (4) serum glutamic oxaloacetic acid transaminase (SGOT), (5) serum vitamin E, and (6) RBC glutathione reductase. Measurements were taken on all six variables preceeding and following both exposure days. Each measurement was treated as a dependent variable in the analysis. Use of multivariate analysis of variance (MANOVA) allowed for evaluation of differences between (a) the air and acid groups, (b) day 1 and 2 measurements, (c) pre- and post-measurements, and (d) all interactions of these effects.

Blood analysis. Serum glutamic oxaloacetic acid transaminase was analyzed on the Centrifichem 400 autoanalyzer by a modified Karmen technique.[8,9] Red blood cell glutathione reductase was assayed by a modified method of Nichoalds:[10] the enzyme was pre-incubated at 0°C for 30 min in the presence of 60 μM flavine adenine dinucleotide (FAD) and the reaction was initiated by the simultaneous addition of 2 mM oxidized glutathione (GSSG) and 0.24 mM nicotinamide adenine dinucleotide phosphate (NADPH, reduced). The method of Nygaard and Rorth[11] was used to measure 2,3-DPG in erythrocytes, and reduced glutathione in serum was measured by the method of Patterson and Lazarow.[12,13] Serum vitamin E was measured by the method of Chaney (unpublished procedure), and lysozyme was measured by the method of Shugar.[14]

A Centrifichem Model 400 centrifugal analyzer and Centrifichem autopipetter (Union Carbide Corporation, Clinical Diagnostics, Rye, NY) were used for assaying SGOT. Manual assays for glutathione reductase, GSH, and 2,3-DPG were run on Cary 118 spectrophotometer; the lysozyme assay was run on a Varian 635 spectrophotometer. Serum glutamic oxaloacetic acid transaminase reagent kits were purchased from the Union Carbide Corporation (Clinical Diagnostics, Rye, NY); 2,3-DPG reagent kits were purchased from Calbiochem (La Jolla, CA); and lysozyme reagent kits were purchased from Worthington Diagnostics (Freehold, NJ).

Alloxan monohydrate, D,L-α-tocopherol, GSSG (free acid, grade III), FAD (disodium salt, grade III), NADPH (tetrasodium salt, Type 1), and GSH (reduced form, 98-100% purity) were purchased from Sigma Chemical Company (St. Louis, MO).

RESULTS

Table 1 provides summary statistics for all six dependent variables, including data from both the air-air and air-acid treatment groups. None of the variables appear to violate assumptions necessary for the analysis of variance.

Table 2 summarizes the MANOVA computed for these data. Each row across the table tests for an effect on any one of the six dependent variables, or any combination of the six simultaneously. The first test listed in Table 2 compares the difference between the air and H_2SO_4 groups on all six variables simultaneously. The second test listed in Table 2 compares the difference between day 1 and day 2. The third test compares the difference between pre-exposure and post-exposure. The remaining tests evaluate the interactions of the exposure and the time of measurement. The important hypotheses tested involved the air-acid difference, none of which were significant. The overall pre- and post-exposure effect, however, was significant ($P < .05$). Of the six univariate tests, only glutathione reductase had a significant effect, as indicated by $P = .001$. No other variable had a pre- or post-exposure P value less than .14. The pre- and post-exposure means for glutathione reductase were 7.27 μmoles/gm Hgb · min, and 7.76 μmoles/gm Hgb · min, respectively. This pre- to post-exposure increase produced the significant P value.

Table 3 provides mean response for all six dependent variables at each point in time for each exposure group. The same data are presented graphically in Figures 1a through 1f. All variables except RBC glutathione indicate nonsignificant changes. These figures support this conclusion, indicating that the exposed and control groups both started close together and remained close together during the experiment. The significant pre- and post-exposure effect for glutathione reductase is easily seen in Figure 1c. When viewing the graph it should be remembered that on day 1 both groups were exposed to air only.

Table 1.—Summary Statistics for All Six Dependent Variables Calculated for Day 1 Exposure

Time	$\bar{X}$*	*SD*†	Minimum Response	Maximum Response	Skewness
			Glutathione mg/100 ml		
Pre-exposure	41.53	4.91	33.60	54.80	0.74
Post-exposure	40.83	5.27	30.00	52.30	−0.03
			Lysozyme μg/ml		
Pre-exposure	10.97	2.89	5.49	17.94	0.34
Post-exposure	10.84	2.71	6.01	17.22	0.33
			Glutathione Reductase μmole/gm Hgb • min		
Pre-exposure	7.08	1.19	4.95	10.21	0.51
Post-exposure	7.70	1.20	5.98	11.52	0.98
			SGOT IU/J		
Pre-exposure	13.2	3.44	7.0	21.0	0.70
Post-exposure	12.9	2.97	9.0	21.0	1.05
			Serum Vitamin E μg/ml		
Pre-exposure	7.40	2.23	3.28	15.90	0.20
Post-exposure	7.43	2.71	3.05	18.14	1.76
			2,3-DPG μmole/ml RBC		
Pre-exposure	4.60	0.63	3.23	6.16	0.04
Post-exposure	4.79	0.79	3.86	7.72	2.07

*$\bar{X}$ = mean

†*SD* = standard deviation

Table 2.—MANOVA Summary for All Six Dependent Variables

Source	Likelihood Ratio	$\tilde{F}$[1]	Numerator *df*	Denominator *df*	*P*
Air/Acid (A)	.935	.32	6	28	.919
Day (D)	.766	1.43	6	28	.239
Pre-post (P)	.652	2.49	6	28	.046*
A × D	.770	1.39	6	28	.252
A × P	.926	.37	6	28	.890
D × P	.886	.60	6	28	.727
A × D × P	.737	1.67	6	28	.166

*Significant at the .05 level.

[1] Rao's *F* approximation for Likelihood Ratio Test.

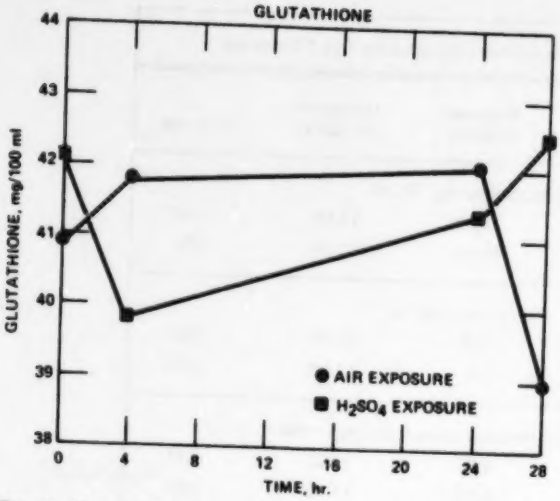

Fig. 1a. Mean GSH response for air and H_2SO_4 groups, pre- and post-exposure on day 1 and day 2.

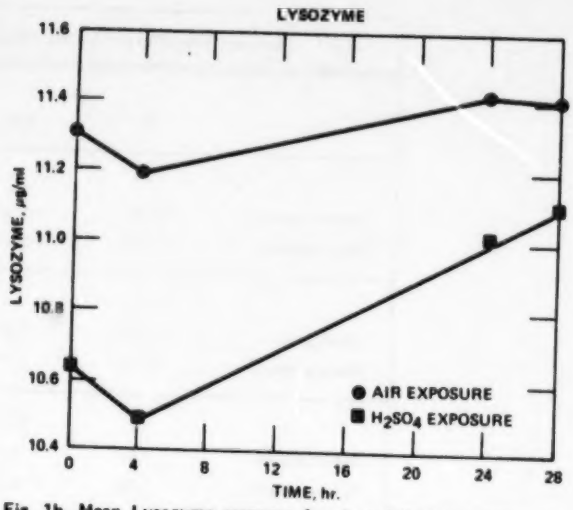

Fig. 1b. Mean Lysozyme response for air and H_2SO_4 groups, pre- and post-exposure on day 1 and day 2.

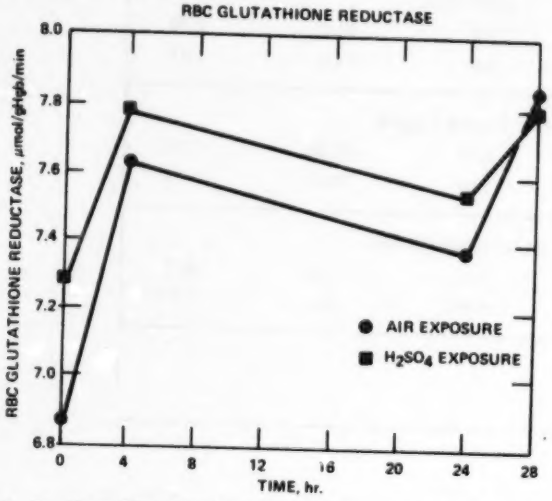

Fig. 1c. Mean RBC GSH Reductase response for air and H_2SO_4 groups, pre- and post-exposure on day 1 and day 2.

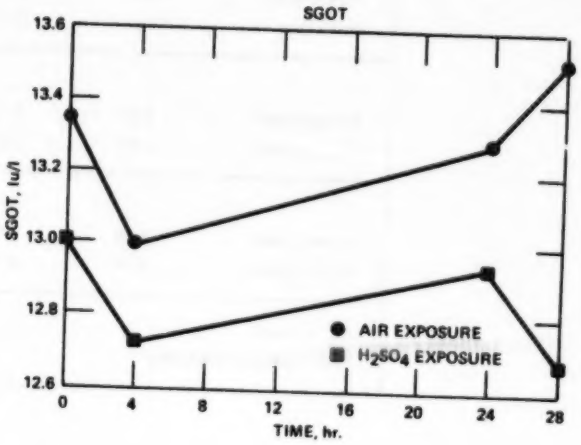

Fig. 1d. Mean SGOT response for air and H_2SO_4 groups, pre- and post-exposure on day 1 and day 2.

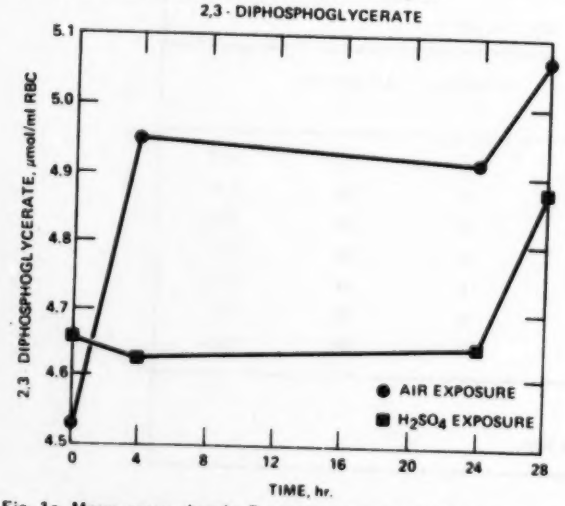

Fig. 1e. Mean serum vitamin E response for air and H_2SO_4 groups, pre- and post-exposure on day 1 and day 2.

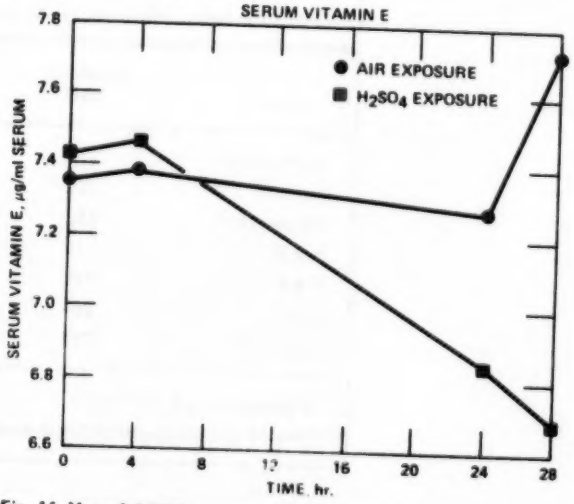

Fig. 1f. Mean 2,3-DPG response for air and H_2SO_4 groups, pre- and post-exposure on day 1 and day 2.

Table 3.—Mean Response on All Six Dependent Variables Estimated from MANOVA

Variable	Treatment	Day 1 Exposure Pre	Day 1 Exposure Post	Day 2 Exposure Pre	Day 2 Exposure Post
Glutathione (mg/100 ml)	air-air	40.93	41.85	42.08	38.99
	air-acid	42.10	39.87	41.36	42.54
Lysozyme (μg/ml)	air-air	11.33	11.21	11.44	11.42
	air-acid	10.63	10.48	11.02	11.12
Glutathione Reductase (μmole/gm Hgb • min)	air-air	6.86	7.63	7.38	7.84
	air-acid	7.29	7.77	7.56	7.81
SGOT (IU/1)	air-air	13.4	13.0	13.3	13.5
	air-acid	13.1	12.7	12.9	12.7
Serum Vitamin E (μg/ml)	air-air	7.36	7.39	7.28	7.74
	air-acid	7.43	7.47	6.86	6.68
2,3-DPG (μmole/ml RBC)	air-air	4.53	4.95	4.93	5.08
	air-acid	4.66	4.63	4.66	4.89

DISCUSSION

The results indicate no effect on the blood parameters measured for one 4-hr exposure of humans to 100 $\mu g/m^3$ (0.033 μM) H_2SO_4 aerosol (0.5 MMD). This study considered biochemical blood parameters that are involved in maintaining cellular reductive detoxification ability, which in turn, protects cellular components from oxidation and also considered the blood lysozyme level which is an indicator of lung tissue damage.

A significant post-exposure increase of GSH-reductase occurred in both the air- and acid-exposed groups for each day of the exposure, thereby eliminating acid exposure as a possible cause of increased GSH-reductase. A plausible hypothesis, not testable with this data, is that subjects were responding to the moderate level of exercise required during the exposure periods.

These results are consistent with the available animal studies.[7,17,18,19] The few effects that are observed normally occur when animals are exposed to high levels of H_2SO_4 (i.e., 0.38 to 4.79 mg/m^3), altered pulmonary functioning is the only resulting effect. The authors suggest that the effects may be confined to the respiratory system. Petering and Shih[15] have suggested that the conversion of sulfites to sulfates is probably a protective mechanism that occurs in the respiratory tract.

The collection schedule for this study was determined by the associated pulmonary function testing schedule, which did not allow detection of an effect unless it was detectable immediately after the exposure was terminated. Because of the noted disadvantage, this research does not support the existence of any effect of H_2SO_4 aerosol exposure on any of the biochemical blood parameters measured.

Theoretically, H_2SO_4 might be expected to initiate a response in the respiratory system due to its low pH (< 1). However, the ammonia released by the respiratory system may well eliminate any effect of inhaled H_2SO_4 aerosol through partial or complete neutralization.[16] Ammonia concentrations ranging from 29 $\mu g/m^3$ to approximately 2,200 $\mu g/m^3$ have been measured in exhaled air of healthy human adults. Stoichiometrically, 1 $\mu g/m^3$ ammonia can convert 5.8 $\mu g/m^3$ H_2SO_4 to ammonium bisulfate and at a concentration of 2.9 $\mu g/m^3$, ammonia can convert H_2SO_4 (5.8 $\mu g/m^3$) to ammonium sulfate.[17] Thus, 35 μg ammonia/m^3 would convert 100 $\mu g/m^3$ H_2SO_4 to ammonium sulfate, and only 17 μg ammonia/m^3 would convert this concentration of H_2SO_4 to ammonium bisulfate. Consequently, the amount of acid aerosol presented was probably neutralized in the respiratory tract. In recent studies utilizing animal species, healthy humans, and human asthmatics there has been no convincing evidence of functional changes upon exposure to ammonium bisulfate and ammonium sulfate.[17] No effects were observed even at concentrations up to one milligram per cubic meter.[17] Thus, it appears that conversion of H_2SO_4 to ammonium bisulfate or ammonium sulfate does constitute an effective defense mechanism.

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